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Expansify™ Cell Culture Methods- HeLa Expansion

Based on internally validated culture conditions and representative experimental data generated using Expansify™ plates

Purpose

This culture guide provides representative conditions for evaluating the effect of initial seeding density and cell source on the expansion of parental HeLa cells obtained from ATCC and CH3 Biosystems. The protocol is based on internally generated experimental data and includes representative seeding conditions, expansion kinetics, cell yields, and harvest timing observed under XDemics validated laboratory conditions. These conditions are intended to identify culture parameters that maximize cell yield while minimizing time to harvest, supporting more efficient biomanufacturing workflows and potentially reducing production costs. Further optimization may be required based on the intended application and desired outcome.

Materials

Prior to use, review the Expansify™ Quick Start Guide and instructional video series in the resources section of the XDemics website.

- Expansify™ 24-Well Plate (Cat. No. 24W-R-01-01) or 1-Well Plate (Cat. No. 01W-R-01-01)
- Cell lines: HeLa (ATCC® CCL-2 ™) and HeLa (CH3 Biosystems, Cat. No. 94A003)
- Single-use glass or polystyrene Pasteur pipettes are required for fluid handling (e.g., CELLTREAT® Polystyrene Pasteur Pipette, Cat. No. 229280)
- Culture medium: DMEM High Glucose (Gibco, Cat. No. 11965092) supplemented with 10% Fisherbrand™ Research Grade Fetal Bovine Serum, Canadian Sourced (Cat. No. FB12999102).
- Phosphate-buffered saline (PBS) Ca^{2+}/Mg^{2+}-free (Gibco, Cat. No. 10-010-023)
- EDTA (0.5 M), pH 8.0, RNase-free (Invitrogen™, Cat. No. AM9260G)
- Dissociation reagent: TrypLE™ Express Enzyme (1×), no phenol red (Gibco, Cat. No. 12604013)
- Extracellular matrix (ECM) coating reagent: Vitronectin (VTN-N) Recombinant Human Protein, truncated (Gibco, Cat. No. A400458)
- Wide-bore P1000 pipette tip (recommended)
  NOTE: Equivalent products from other manufacturers may be substituted for the culture medium, FBS, Ca^{2+}/Mg^{2+}-free PBS, EDTA, TrypLE™ cell dissociation reagent, vitronectin coating reagent, and wide-bore P1000 pipette tips listed above. All other materials should be used as specified, including the listed manufacturer and catalog number.

Experimental Procedure

Day -1: ECM Coating

1. Prepare ECM-coated Expansify™ plates according to **Expansify™ Technical Note - Extracellular Matrix (ECM) Coating of Expansify™ Plates**.

Day 0: Culture Initiation

1. Aspirate the ECM coating solution from the Expansify™ 24-Well Plate using the 0.25 mL SureShelves™.
2. Immediately add 1 mL of complete culture medium to each well.
3. Inspect the bottom of the niches for trapped air bubbles. If air bubbles are visible, centrifuge the plate at 200 × g for 1 minute using an appropriate Expansify™ plate or deep-well plate as a balance. Confirm that the niches are fully wetted before proceeding.
   NOTE: Following ECM coating, the culture surface is typically already wetted, and centrifugation may not be necessary. However, occasional air bubbles may remain or become trapped within the niches. These bubbles can prevent complete filling of the niche and reduce the surface area available for cell attachment and growth.
4. Seed HeLa cells at $2.5 \times 10^4 – 5 \times 10^5$ cells/cm^2 into the desired wells of the Expansify™ 24-Well Plate.
5. Adjust the final culture volume to 4.5 mL per well.
6. Following seeding, gently homogenize the cell suspension to promote uniform cell distribution and settling throughout the culture niches.
7. Monitor and image cultures as needed throughout the culture period (e.g., Days 1–7).

Days 3, 4, 5, and 6: Medium Exchange

1. Perform complete medium exchanges according to the cell source and seeding condition:
   1. CH3 Biosystems HeLa cells:
      i. 2.5×10^5 cells/well: Days 4 and 5.
      ii. 1×10^6 cells/well: Days 3, 4, and 5.
   2. ATCC HeLa cells:
      i. 1×10^5 cells/well: Day 5.
      ii. 2×10^5 cells/well: Day 5.
      iii. 4×10^5 cells/well: Days 4 and 6.

   NOTE: No medium exchange is performed for the 5×10^4 cells/well CH3 Biosystems or ATCC conditions.
2. Keep the plate level on the work surface and aspirate the spent medium using the 0.25 mL SureShelves™. Add 4.5 mL of fresh complete medium using the 0.25 mL or 1 mL SureShelves™.

Days 6 and 7: Cell Harvest

1. Harvest cells according to the cell source and designated harvest day:
    a. **CH3 Biosystems HeLa cells:** Day 6; **ATCC HeLa cells:** Day 7
        i. **Wash the culture:** Keep the plate level on the work surface. Aspirate the spent medium using the 0.25 mL SureShelves™. Add an excess volume of wash buffer, > 2 mL per well, such as 1× PBS (Ca^{2+}/Mg^{2+}-free) supplemented with EDTA to a final concentration of 5 mM, using the 0.25 mL or 1 mL SureShelves™, then aspirate the wash buffer using the 0.25 mL SureShelves™.
        ii. **Initiate cell dissociation:** Add TrypLE™ Express Enzyme (1×) to each well to be harvested according to the anticipated cell number, estimated based on the expected doubling time and duration of culture:
            1. **≤ 2×10^6 cells/well:** 1 mL
            2. **> 2×10^6 to < 1×10^7 cells/well:** 2–2.5 mL
            3. **≥ 1×10^7 cells/well:** 2.5–3 mL

            Gently mix up and down 2–3 times using a P1000 wide-bore pipette tip to distribute the enzyme throughout the well, then incubate at 37°C and 5% CO_2 for 7–10 minutes.
        iii. **Assess dissociation:** Remove the plate from the incubator and gently mix the culture using a P1000 wide-bore pipette tip. Examine the cells under a microscope to assess dissociation. If a uniform single-cell suspension has not been achieved, return the plate to the incubator for an additional 7–10 minutes, then repeat the gentle mixing step. Avoid excessive mixing or mechanically forcing dissociation, as this may increase shear stress and reduce cell viability.

            NOTE: The recommended TrypLE™ Express Enzyme (1×) volumes and incubation conditions are based on XDemics' internal validation. Under these conditions, a uniform single-cell suspension should typically be achieved within a cumulative incubation time of approximately 30 minutes at the highest cell densities evaluated in Expansify™, with shorter incubation times generally sufficient at lower cell densities. If additional dissociation is required, repeated incubation and gentle mixing steps may be performed, and additional TrypLE™ Express Enzyme (1×) may be added as needed. Additional incubation beyond 30 minutes should be minimized to limit unnecessary enzymatic exposure and preserve cell integrity and viability.
        iv. **Collect the cells:** Collect the resulting cell suspension for downstream processing, analysis, or further expansion.

            NOTE: TrypLE™ Express Enzyme (1×) does not require neutralization. Following cell dissociation, complete culture medium may be added to dilute residual enzyme activity as appropriate for downstream processing. Record the final harvest volume prior to downstream processing.

Performance Summary

Data represent two independently harvested wells per condition at each time point (n = 2), with separate wells destructively harvested at each time point.

CH3 Biosystems HeLa cells: Cells expanded consistently over the 6-day culture period. Cultures seeded at 50,000 and 250,000 cells/well exhibited similar mean doubling times of 26.52 and 27.87 h, respectively, consistent with the 24–26 h doubling time measured in internal T-flask experiments. Cultures seeded at 1.0×10^6 cells/well exhibited a slower mean doubling time of approximately 33.88 h. Cell viability remained >91% at all measured time points, as assessed by AOPI staining using the Cellaca MX cell counter with the built-in "Cell Lines" assay.

ATCC HeLa cells: Cells also expanded consistently across the tested seeding densities, with a mean doubling time of 41.83 ± 2.20 h. Growth rate showed no clear dependence on initial seeding density and was slower than the 29–31 h doubling time measured in internal T-flask experiments. Cell viability remained >94% at final harvest across all conditions, as assessed by AOPI staining using the Cellaca MX cell counter with the built-in "Cell Lines" assay.

Overall, both parental HeLa cell sources expanded successfully in Expansify™ cultureware. Growth kinetics varied by cell source and, for CH3 Biosystems HeLa cells, by initial seeding density, supporting cell-source qualification and seeding-density optimization to minimize time to target cell yield and improve manufacturing scheduling.

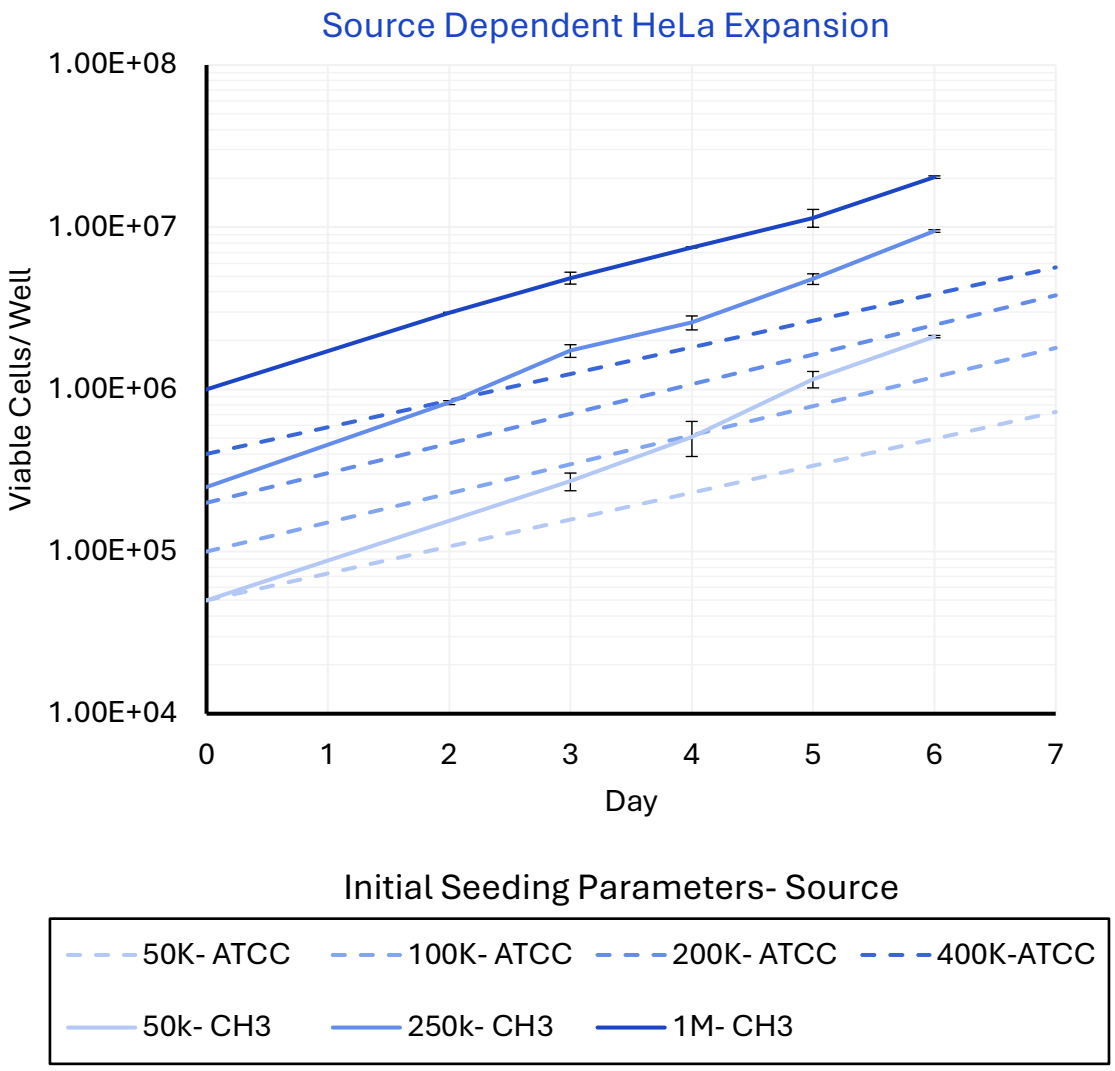
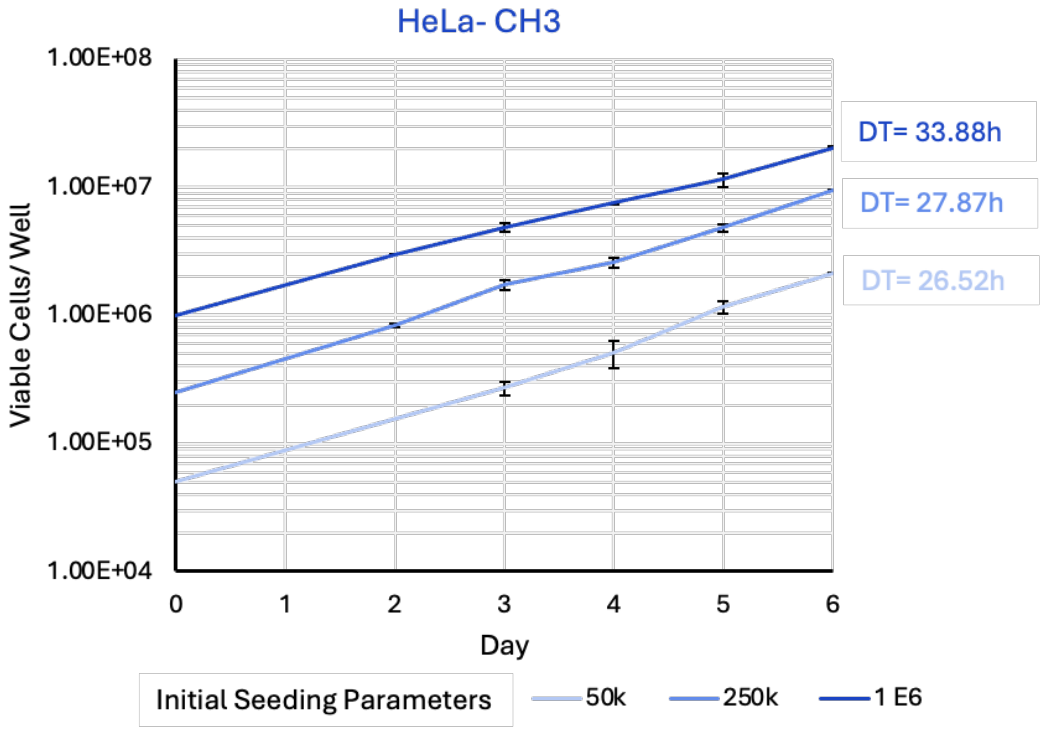
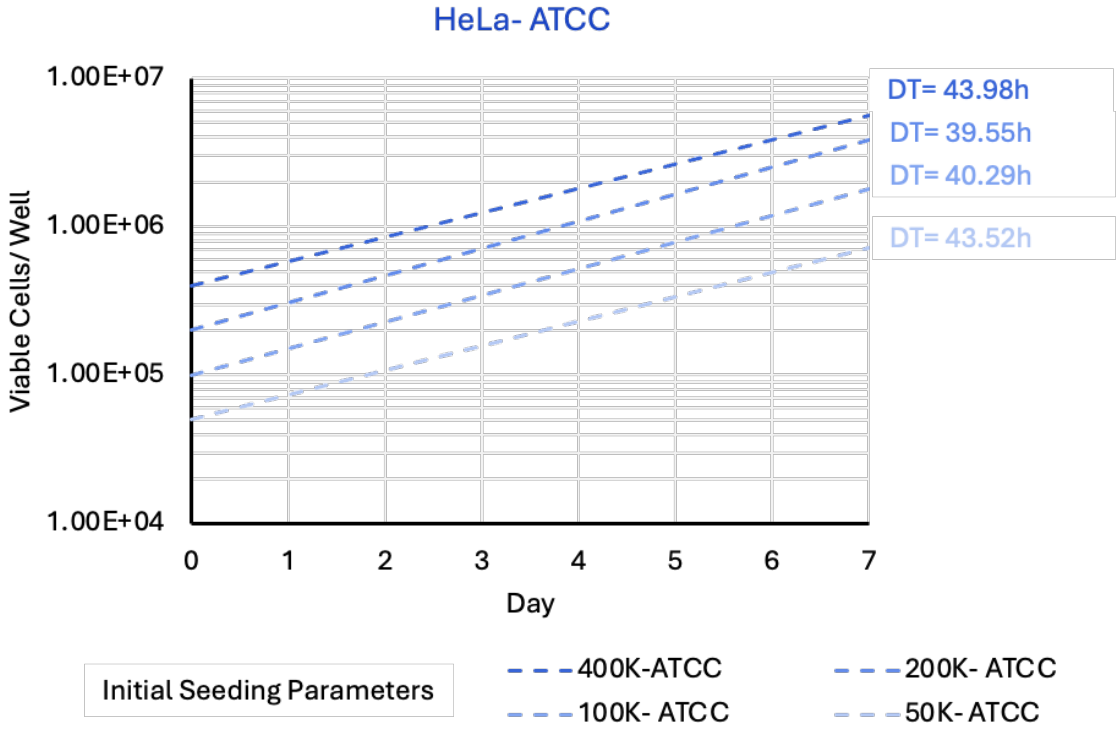


Figure 1. Growth kinetics of parental HeLa cells from ATCC and CH3 Biosystems. ATCC-sourced HeLa cultures represent final harvest measurements for each tested seeding condition (n = 1). CH3 Biosystems-sourced HeLa cultures were harvested at daily time points from Days 3-6, with duplicate measurements at each time point (n = 2). Growth curves for both cell sources are shown together to facilitate comparison of expansion kinetics across cell sources and initial seeding conditions.


HeLa- CH3
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
Viable Cells/ Well
0
1
2
3
4
5
6
Day
DT= 33.88h
DT= 27.87h
DT= 26.52h
Initial Seeding Parameters
50k
250k
1 E6


HeLa- ATCC
1.00E+07
1.00E+06
1.00E+05
1.00E+04
Viable Cells/ Well
0
1
2
3
4
5
6
7
Day
DT= 43.98h
DT= 39.55h
DT= 40.29h
DT= 43.52h
Initial Seeding Parameters
400K-ATCC
200K- ATCC
100K- ATCC
50K- ATCC